quantitatively probe PKR dimerization upon binding to RNA. The fluorescence anisotropy of a probe placed near the kinase dimer interface decreases upon PKR binding to an activating 40 bp dsRNA due to depolarization induced by homo-FRET. Thus, the kinase domains form a dimer when two PKR monomers bind to this dsRNA. Surprisingly, several non-activating dsRNAs also induce dimerization, suggesting that PKR dimerization is necessary but not sufficient for activation.

2004-Pos Board B141
Quantitative DNA Binding, Looping, and Compaction Properties of the HIV-1 Viral Protein R
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Human immunodeficiency virus type 1 (HIV-1) Viral protein R (Vpr) is packaged into virions (~200 molecules) and is essential for viral replication. While several *in vivo* functions have been attributed to Vpr, one primary function is believed to be transport of the HIV-1 pre-integration complex into the nucleus. Because the nuclear pore diameter is approximately 25 nm, the DNA must be highly compact in order to enter the nucleus. To understand the mechanism by which Vpr may facilitate this nuclear transport, we combine single molecule stretching and atomic force microscopy (AFM). We measure the DNA binding affinity of Vpr for the first time. We then investigate the ability of Vpr to both bind and compact DNA. To do this, we hold DNA at low force for fixed times, allowing it to form loops and other compact structures. We find that the timescale required for the formation of large loops due to protein-DNA bridging is several minutes. In contrast, by holding the DNA molecule at a constant force of 15 pN and measuring its length change, Vpr can also actively compact DNA on the timescale of tens of seconds (15 $\pm$ 2 s), representing a different compaction process involving DNA shortening likely due to Vpr binding alone. The persistence length determined from AFM at a low concentration is much longer than that of bare DNA, suggesting that Vpr forms shorter but more rigid structures upon binding DNA. AFM images also demonstrate DNA bridging between strands, consistent with the looping observed in optical tweezers experiments. These results support a model in which Vpr translocates the viral DNA into the nucleus by forming compact structures mediated by protein-DNA and protein-protein interactions.

2005-Pos Board B142
Repetitive Single-Molecule FRET Fluctuations upon T4 Gene 32 Protein Binding to Single-Stranded DNA
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Bacteriophage T4-coded gene 32 protein (gp32) is an essential component of the T4 DNA replication, recombination, and repair systems. Gp32 is a single-stranded DNA binding protein that binds cooperatively to single-stranded (ss) DNA templates exposed by the function of the T4 primosome helicase, and configures these templates for efficient use by the replisome. To this end gp32 binding helps melt out transiently formed ssDNA secondary structures, protects exposed ssDNA sequences from nucleases, and helps to integrate the functions of the other components of the replication complex. It has been well studied by bulk biochemical methods for about 40 years. However, many aspects of the detailed mechanisms – and especially the dynamics – of the interactions of gp32 with both double-stranded (ds) and single-stranded DNA are still not well studied or well understood.
We have used single-molecule Förster Resonance Energy Transfer (sm-FRET) methods to monitor the cooperative and non-cooperative binding of gp32 to the ssDNA sequences of model DNA replication forks, and have shown that the observed binding dynamics depend significantly on gp32 concentration, salt concentration and ssDNA segment length. Upon addition of 1 μM concentrations of gp32 into a sample chamber containing tethered single molecule replication fork constructs, we observed the appearance of 'repetitive FRET fluctuations' of the individual DNA molecules (>70% of the molecules per imaging area) on a 100 ms timescale. We noted also that these repetitive FRET fluctuations were substantially less prominent under the conditions of tight and fully cooperative gp32 protein binding. We have used ensemble and single-molecule fluorescence approaches to probe the dynamics of these gp32-ssDNA interactions in detail, and will discuss possible molecular interpretations of these smFRET fluctuations in terms of potential reaction intermediates and association-dissociation pathways in real time.

2006-Pos Board B143
How MeCP2 and R.DpnI Proteins Recognize Methylated DNA
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DNA methylation plays a major role in organismal development and the regulation of gene expression. Methylation of cytosine bases and its cellular roles in eukaryotes are well established, as well as methylation of adenine bases in bacterial genomes. Here, we present results from molecular dynamics simulations, alchemical free energy perturbation, and MM-PBSA calculations to explain the specificity of the R.DpnI enzyme for binding to adenine-methylated DNA in both its catalytic and winged-helix domains. We find that adenine-methylated DNA binds more favorably to the catalytic subunit of R.DpnI (−4 kcal/mol) and to the winged-helix domain (−1.6 kcal/mol) than unmethylated DNA.
In particular, N6-adenine methylation is found to enthalpically stabilize binding to R.DpnI. In contrast, C5-cytosine methylation stabilizes binding to the MBD domain of the MeCP2 entropically with almost no difference in binding enthalpy.

2007-Pos Board B144
DNA Looping and Genome Architecture: How Proteins can Connect and Organize Chromosomes
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The control of gene expression sometimes entails the folding of DNA into looped structures mediated by the binding of protein. Although the literature abounds with examples of single DNA loops induced by the attachment of sequentially distant genetic elements on a common protein core, recent studies have demonstrated the occurrence of multiple loops formed by three or more remote, protein-anchored sites. For example, the Escherichia coli Gal repressor has the ability to form oligomeric structures leading to higher-order helical protein pathways that can secure multiple chromosomal connections. Moreover, several novel experimental investigations have highlighted the role of bridging proteins, such as the macrodomain Ter protein (MatP) and the histone-like structuring protein (H-NS), in chromosome condensation and organization. These proteins are thought to be able to bridge distant DNA sites and to participate in the folding of the bacterial genome. We are examining the entanglement of DNA loops that attach to such proteins with the help of a novel energy minimization method associated with traditional Monte Carlo approaches. We focus on the multiple loops that can be induced by oligomeric Gal assemblies and report the relevant energy landscapes and topological and statistical properties as functions of the number of Gal repressors and the chain lengths of the different loops. In addition, we take advantage of the fact that our optimization method accounts for the presence along DNA of bound ligands to reveal how the binding of architectural proteins (e.g., the Escherichia coli histone-like HU protein) can ease or suppress the formation of such loops. Finally, we examine the influence of MatP and H-NS on the conformation and fluctuations of DNA minicircles to understand how these proteins may contribute towards the formation of topological domains.

2008-Pos Board B145
Common Aspects of G-Quadruplex Destabilization among Helicases and Single Stranded DNA Binding Proteins
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Various helicases and single-stranded DNA (ssDNA) binding proteins are known to destabilize G-quadruplex (GQ) structures, which otherwise result in genomic instability. Bulk biochemical studies have shown that Bloom helicase (BLM) unfolds both intermolecular and intramolecular GQ in the presence of ATP. Using single molecule FRET, we show that binding of BLM to ssDNA in the vicinity of an intramolecular GQ leads to unfolding of the GQ in the absence of ATP. We show that the efficiency of BLM-mediated GQ unfolding correlates with the binding stability of BLM to ssDNA overhang, as modulated by the nucleotide state, ionic conditions, overhang length, and overhang directionality. A related protein WRN showed similar activity to BLM. These results are surprisingly similar to those we observed on interactions of ssDNA binding protein Replication Protein A with GQ, which also does not require ATP or enzymatic activity to unfold GQ. These similarities point out to common features of GQ destabilization mechanisms of helicases and ssDNA binding

proteins, in which binding of the protein is what initiates and in some instances is adequate to unfold the GQ.

2009-Pos Board B146

Dynamic Interactions between DNA and the T4 Single-Stranded Binding Protein gp32: Multi-Dimensional Correlation Analysis of Microsecond Single-Molecule FRET and Linear Dichroism Fluctuations

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Protein-nucleic acid interactions are of central importance in genome-regulatory processes. The DNA replication system of the T4 bacteriophage is an excellent model system to study DNA replication in higher organisms, since the T4-coded replication machinery utilizes many of the same components. Here we present studies of the kinetics of binding of gp32, the single-stranded (ss) DNA binding protein of the T4 system, whose roles include the functional integration of the other components of the T4 replication complex as well as protecting the transiently-exposed single-stranded DNA template sequences from DNA nucleases and melting out unfavorable secondary structures potentially formed by the lagging strand during DNA replication. We have performed single-molecule measurements of internally-labelled Cy3/Cy5 labeled primer-template DNA constructs in the presence of gp32 by simultaneously monitoring single-molecule Förster resonance energy transfer (smFRET) and single-molecule fluorescence-detected linear-dichroism (smFLD) on the microsecond time scale. smFRET measurements probe the distance between the fluorophores, while smFLD measurements are sensitive to local orientations of the Cy3-labeled sugar-phosphate backbones of the DNA construct. Multiple transient FRET states are observed when adding gp32 protein to tethered model replication fork DNA constructs, permitting us to track the dynamics of millisecond interconversions between various configurations of the gp32 / ssDNA system. The distribution of FRET states changes as a function of gp32 concentration, suggesting that we may be monitoring fluctuations that reflect changes associated with the progression from isolated to cooperative gp32 binding. We apply a multi-dimensional correlation function analysis to our microsecond-resolved smFRET and smLD data to reveal statistically relevant mechanistic information about the reaction pathways of the ssDNA-gp32 system at the replication fork.

2010-Pos Board B147

Exploration of Cytosine Methylation Effects on Protein-DNA Binding

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Bisulfite sequencing has been employed to great effect to identify DNA methylation changes between different tissues, through stem cell differentiation, and even in cancer development. However, there is an ever increasing problem of assessing the functional importance of these methylation changes- though work has demonstrated that methylation affects protein-DNA interaction, a clear and comprehensive delineation of the strength of these effects is lacking. We employed a modified chromatin immunoprecipitation method combined with bisulfite sequencing to identify the distribution of methylation patterns present in bound DNA. We used both synthetically generated in vitro samples for an unbiased measurement and samples derived from cell lines for physiologically relevant patterns. Our library preparation methodology uses magnetic bead ChIP (Life Technologies) combined with methylated hairpin adapters (NEB) and low-input bisulfite methods (Zymo Research) with custom modifications. We then sequenced these libraries on an Illumina MiSeq for the long-read length offered (2x300). The results were compared to the input methylome to determine the relative frequency of methylation patterns bound by protein. In our initial work, we examined the methylation patterns of DNA bound by CTCF and MeCP2. The number of methylated locations required to affect protein-binding was measured, as well as the frequency of binding versus a known binding sequence. We specifically examined DNA upstream and downstream of the known binding motifs, to determine non-local effects of methylation on protein-DNA affinity.

2011-Pos Board B148

Molecular Mechanism of Processive 3' to 5' RNA Translocation in the RNA Exosome Complex

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3' to 5' degradation of a wide range of RNA molecules is performed by the exosome complex, as a key part of cellular quality control. Recent structural studies of this complex revealed that ssRNA is channeled through its multisubunit ring-like core into the active site tunnel of its exonuclease subunit Rrp44. Rrp44, both alone and in the exosome, processively cleaves RNA nucleotides one at a time, without consuming the energy of ATP, and releases a final 3-5 nucleotide product. We use molecular dynamics simulations and free energy calculations to identify the factors that enable processivity of RNA translocation and cleavage in the exosome complex. Our simulations reveal large and favorable free energies of RNA transfer from solution into the active site of Rrp44. The free energy profiles that characterize RNA translocation within the active site of Rrp44 are found to be dependent on the length of the RNA strand. While RNA strands formed by 5 nucleotides or more have downhill free energy profiles along the translocation coordinate towards the cleavage site, a 4-nucleotide RNA has a free energy barrier along the same coordinate, potentially leading to incomplete cleavage of ssRNA and the release of short (3-5) nucleotide products. Furthermore, dynamic insights gained from the performed simulations help elucidate the concerted nature of RNA translocation through the exosome complex.

2012-Pos Board B149

Probing DNA Bending Kinetics by yNhp6A with Ultrafast Temperature Jump Spectroscopy

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The yeast Nhp6A protein is a member of the eukaryotic HMGB family of chromatin factors that enhance apparent DNA flexibility in many cellular processes. yNhp6A exhibits no sequence-specificity but binds to DNA with 1-10 nM affinity, sharply bending the DNA by >60° at the binding site. The kinetic mechanism by which this DNA deformation occurs remains unclear. It is not known whether the protein first binds weakly to unbent DNA and then deforms the DNA or if partially bent DNA conformations are thermally accessible and are ''captured'' and stabilized by the bound protein. The limited (tens-of-milliseconds) time resolution of previous kinetics studies was insufficient to observe the dynamics of DNA deformations in this complex. Here, we report time-resolved FRET measurements on yNhp6A bound to an 18-bp DNA oligomer labeled at each end with Cy3/Cy5, in response to a laser temperature-jump (T-jump) perturbation. The us temporal resolution of T-jump, together with ionic-strength and concentration dependence of equilibrium FRET and anisotropy measurements, helps reveal some of the microscopic kinetics steps. Equilibrium measurements with varied Nhp6A-DNA concentrations indicate that, at 250mM NaCl, the decrease in FRET with increasing temperature, in the range 15-60°C, is from unimolecular unbending of DNA. Kinetics traces measured under these salt conditions are single-exponential with relaxation times ranging from ~400 us (at 60°C) to ~1ms (at 45°C). At lower [salt] < 200mM NaCl, and at temperatures >65°C, bi-exponential kinetics are observed, likely corresponding to unimolecular bending/unbending, followed by dissociation of the complex and concurrent DNA melting. These results represent the first observation of DNA bending/unbending dynamics in complex with a nonspecific DNA-binding protein, and are an important step towards a more comprehensive understanding of the kinetic mechanisms of DNA binding and bending interactions by this class of proteins.

2013-Pos Board B150

Single-Molecule DNA Melting Bubble Formation and Single-Strand Binding Protein Interaction

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Exposed single-stranded DNA (ssDNA) in cells is threatened by degradation from nucleases (DNases) and prone to form secondary structures, which is why cells use single-strand binding proteins (SSB) to protect ssDNA. The most common example is exposure of the Okazaki fragments during replication, or double-stranded DNA melting induced by negative superhelicity, i.e. underwinding of double-stranded DNA caused by replication or transcription. Magnetic tweezers are the tool of choice to mimic and study the influence of superhelicity on DNA structure at the single-molecule level owing to the direct ability to apply torsional stress to double-stranded DNA. Here, we study the conditions of ssDNA exposure and formation from double-stranded DNA (dsDNA) samples with negatively supercoiled single DNA molecules with magnetic tweezers.